

## **COMPARISON OF ORNAMENTAL AND WILD SALT CEDAR (*TAMARIX* SPP.) ALONG EASTERN MONTANA, USA RIVERWAYS USING CHLOROPLAST AND NUCLEAR DNA SEQUENCE MARKERS**

Author(s): John F. Gaskin and David J. Kazmer

Source: Wetlands, 26(4):939-950. 2006.

Published By: The Society of Wetland Scientists

DOI: 10.1672/0277-5212(2006)26[939:COOAWS]2.0.CO;2

URL:

<http://www.bioone.org/doi/full/10.1672/0277-5212%282006%2926%5B939%3ACOOAWS%5D2.0.CO%3B2>

---

BioOne ([www.bioone.org](http://www.bioone.org)) is an electronic aggregator of bioscience research content, and the online home to over 160 journals and books published by not-for-profit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/page/terms\\_of\\_use](http://www.bioone.org/page/terms_of_use).

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

## COMPARISON OF ORNAMENTAL AND WILD SALT CEDAR (*TAMARIX* SPP.) ALONG EASTERN MONTANA, USA RIVERWAYS USING CHLOROPLAST AND NUCLEAR DNA SEQUENCE MARKERS

John F. Gaskin and David J. Kazmer

United States Department of Agriculture, Agricultural Research Service  
Northern Plains Agricultural Research Laboratory, Pest Management Research Unit  
1500 North Central Avenue  
Sidney, Montana, USA 59270  
E-mail: jgaskin@sidney.ars.usda.gov  
dkazmer@sidney.ars.usda.gov

**Abstract:** Saltcedars (*Tamarix ramosissima*, *T. chinensis*, and their hybrids) have invaded riverways and lakeshores across the western USA and northern Mexico. In Montana, ornamental plantings of saltcedar have been hypothesized, to varying degrees, to be the origin of nearby, wild populations. To examine this hypothesis, we compared chloroplast and nuclear DNA sequences from 36 ornamental and 182 wild saltcedars from Montana, North Dakota, and Wyoming, USA. We found that ornamental and wild population genotype frequencies were highly dissimilar. Also, genotype frequencies of hypothetical propagule populations under scenarios of random mating, self-fertilization, and clonal reproduction in the ornamental population were highly dissimilar to the genotype frequencies of the wild populations. Assignment tests indicated that the majority of wild genotypes originated from other wild plants, not from ornamental plants. However, ornamental plants could not be excluded as contributors to wild populations because all chloroplast and nuclear haplotypes found in the ornamental plants were found at some frequency in the wild. These findings suggest that while ornamental saltcedars are not the sole source of wild saltcedar, they do have potential to contribute genetic material to an invasion or re-establish a population after existing wild saltcedars are removed.

**Key Words:** tamarisk, ornamental, horticulture, invasion, naturalized, northern Great Plains

### INTRODUCTION

The majority of woody plant invasions in the USA originated from deliberate, rather than accidental, plant introductions (Reichard 1997). *Tamarix* spp. (family Tamaricaceae) are woody shrubs or trees that were brought to North America in the 1800s as ornamental plants and for wind and erosion control (Horton 1964). *Tamarix ramosissima* Ledeb., *T. chinensis* Lour., and their hybrids (Gaskin and Schaal 2002), commonly called saltcedars, have formed large invasions throughout many riparian areas of the western USA and northern Mexico (Robinson 1965, Ríos and Garcia 1998, Stenquist 2000). They are now declared noxious weeds in many regions of the western United States (Rice 2005). Even with their regional noxious status, saltcedars are still used as ornamental plants and can be purchased at plant nurseries in the USA and Canada.

Many plant invasions have been started from seed or clonal material derived from nearby ornamental

plantings (Reichard 1997, Mack and Erneberg 2002). Studies have also found many examples of hybridization and introgression of ornamental or crop species and related plants found in the wild (e.g., Ellstrand 2003, Jenczewski et al. 2003, Chapman and Burke 2006). In a few cases, there has been a need to determine if invasions can originate from putatively sterile plants (e.g., Anderson and Ascher 1993) or from plant strains used specifically for commercial purposes (e.g., Wiedenmann et al. 2001). The outcome of this type of research is important evidence regarding the safe use of certain plants as ornamentals.

A recent study of saltcedar distribution in northern Montana (Pearce and Smith 2003) suggested that, in some cases, ornamental plants may have played a role in starting local wild populations. In these cases, the ornamental plants were older than plants in the wild and no other seed sources were nearby. A specific example cited was the Musselshell River, which contains an estimated 24,500 saltcedars ranging from seedlings to plants approximately 25 years old. Dispersal mechanisms other than

---

Authors contributed equally to this manuscript.

localized spread of seed from ornamentals were suggested for other areas in the study, including long distance seed movement by wind and water, earth-moving equipment, and trailered boats. Earlier genetic research, which included a small number of ornamental saltcedars from other areas in the western USA, showed that, in most cases, the ornamental and wild saltcedar plants have different chloroplast haplotypes, although the ornamental haplotype was present in the wild in rare cases (Gaskin 2003).

Genetic markers, when there is adequate variation, make it possible to acquire information concerning species or ecotype identity, phylogenetic relationships, and likely origins of invasive organisms (e.g., Cornuet et al. 1999). Such markers are especially useful when few or no reliable morphological traits are present for determining identity (such as between wild and ornamental saltcedars) or when morphological traits are likely to be influenced by environmental variation. Earlier studies on the *Tamarix* invasion (Gaskin and Schaal 2003) have found interspecific variation in the *trn* S-G chloroplast region and intraspecific variation in the fourth intron of phosphoenolpyruvate carboxylase (*PepC*) (Gaskin and Schaal 2002), a low-copy nuclear gene (Panstruga et al. 1995). Here, we use these chloroplast and nuclear DNA sequence markers to determine the genotypes of ornamental and wild saltcedars in the Montana region, to compare these genotypes to those of other specimens from the Western USA and Eurasia, and to determine if any of the genetic markers that characterize Montana's ornamental saltcedars are present in nearby or downstream wild saltcedars.

Several species of *Tamarix* were introduced to the east coast of the USA as early as 1823, and by the 1850s could also be purchased in the southwestern part of the country (Horton 1964). Naturalization of saltcedar occurred decades later, with populations of *T. gallica* L. showing up on the USA Gulf Coast in the 1870s, and *T. pentandra* Pall. (correctly named *T. ramosissima* (Baum 1978)) becoming common along southwestern rivers during the 1890s and 1900s (Horton 1964). Today, the largest *Tamarix* invasion (*T. ramosissima*, *T. chinensis*, and their hybrids) encompasses hundreds of thousands of hectares throughout the western USA (Robinson 1965, Stenquist 2000).

The northern Great Plains invasion is more recent than that of the southwest USA, with establishment on major rivers of Montana (Bighorn, Powder, and Yellowstone) not being recorded until the early 1960s (Robinson 1965). These populations may have been established by seed carried from pre-existing

upriver sources in Wyoming that were established earlier (1950 on the Bighorn River: Pearce and Smith 2003). The Musselshell River in central Montana also contains wild saltcedar populations, although ages of current trees set an establishment date of no later than 1978 (Pearce and Smith 2003). Unlike the other major rivers in Montana that contain saltcedar, the Musselshell has no upstream or nearby wild sources for seed, and thus, pre-1978 ornamental plantings of saltcedar near the river are viewed as a likely source of the wild plants (Pearce and Smith 2003).

The saltcedar species involved in this study, *T. ramosissima* and *T. chinensis*, are generally considered morphologically very similar (Crins 1989, but see also Baum 1978), especially in light of their high rates of hybridization in the USA (Gaskin and Schaal 2002). Commercial *Tamarix ramosissima* cultivars include names such as 'Pink Cascade,' 'Rosea,' 'Rubra,' and 'Summer Glow.' 'Pink Cascade' is known for its dense, dark pink plumes of flowers (due to mostly compound, not simple, inflorescence racemes) and finely textured blue-gray foliage (J. Gaskin pers. observ.). The cultivars can differ in the intensity of flower color, density of inflorescences, and foliage color. Wild *T. ramosissima* is also highly variable in flower color, ranging from the deep red to white, and on a single plant, both simple and compound racemes can be found, making the density of inflorescences also highly variable. Wild saltcedar foliage color varies from dark green to the blue-gray found in the 'Pink Cascade' cultivar (J. Gaskin pers. observ.). All other characters that are important for distinguishing *Tamarix* species (e.g., number of petals and anthers, leaf morphology, nectary disk morphology, and habit (Baum 1978)) do not differ consistently between ornamental and wild plants of the same species (Gaskin 2003).

Saltcedar is able to reproduce sexually and also clonally from woody fragments (Brotherson and Field 1987). There are anecdotal reports of self-fertilization in *Tamarix* (*T. parviflora* DC. producing viable seed, J. Gaskin unpublished data), and also of inability to self-fertilize (a *T. chinensis* cultivar, R.G. Means, pers. comm. in Crins 1989). Putatively sterile ornamental saltcedars are distributed by various nurseries (pers. comm. in Pearce and Smith 2003, page 217), but assumptions of sterility in ornamental cultivars should be viewed with caution (e.g., loosestrife cultivars (*Lythrum* spp.): see Anderson and Ascher 1993, Lindgren and Clay 1993, Ottenbreit and Staniforth 1994).

To start a new population from an isolated ornamental plant, either woody fragments broken

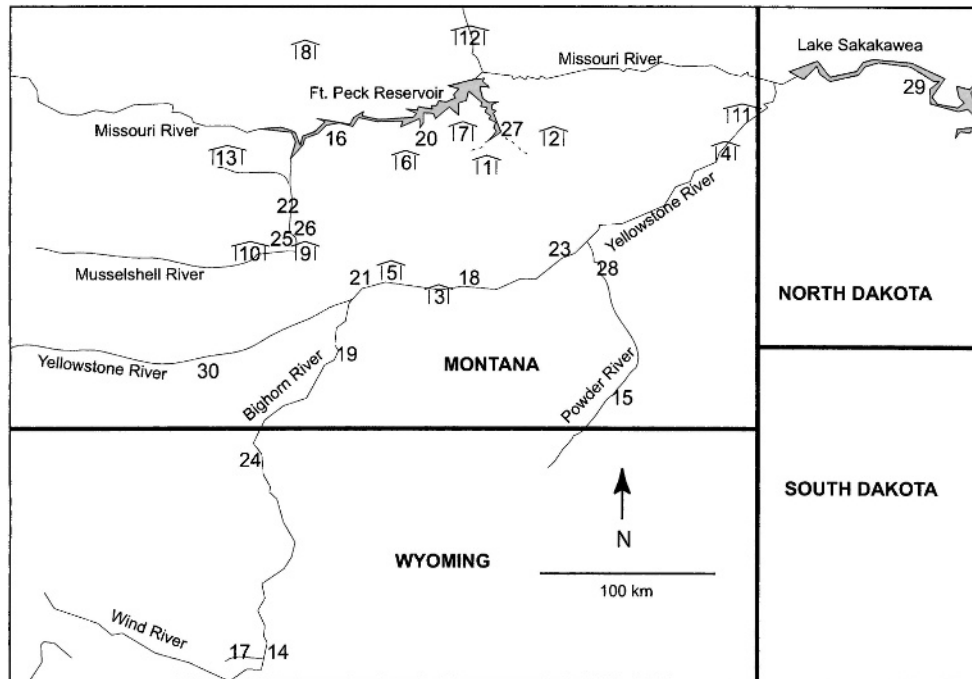


Figure 1. Saltcedar (*Tamarix* spp.) collection locations in the eastern Montana region, USA. Numbers correspond to locations listed in Table 2. The house symbol surrounding a number indicates a location where one or more ornamental plants were sampled. Numbers without houses indicate wild populations sampled.

off and washed downstream must clonally propagate, which is possible for saltcedar, or the plant must self-fertilize. Plants produced from woody fragments would be genetically identical to the original source plant, while progeny derived from self-fertilization would be genetically very similar to the parent plant, with the only variation being due to recombination. If multiple ornamental plants were the only seed source for nearby wild plants, we would expect them to be genetically similar to the wild plants, after allowing for new genotypic combinations through sexual reproduction.

If the ornamental plants were not the original source of wild plants but contributed genetic material to existing wild populations through ovules, pollen, or clonally propagated woody fragments, we would expect to find some level of genetic material specific to ornamental plants existing in the wild. The possibility of ornamental and wild saltcedars crossing is untested. Ornamental plants that we sampled may not have all originated from commercial sources, as there is anecdotal evidence of saltcedar plants or cuttings being removed from the wild and planted in gardens (pers. comm. in Pearce and Smith 2003, also A. Adler, Rosebud Co., MT Weed District, pers. comm.). Alternatively, if the ornamental and wild plants have had no influence on each other, no trace of the genetic

material that is specific to ornamental plants should be present in the nearby wild plants.

The origination of the Musselshell River wild populations from sources other than ornamental plantings is not unreasonable. Pearce and Smith (2003) recognized wind, water, and construction and recreational equipment as possible dispersal mechanisms in other areas. Saltcedar seeds are very small (0.5 mm in length) and light (less than 0.0001 g (Wilgus and Hamilton 1962)), with a 2 mm tuft of hairs (a coma) forming at the apical end, which could allow them to stick to birds, mammals, machinery, or become airborne or waterborne for potentially long distance dispersal. The shortest air distance from the older wild populations on the Yellowstone River to the Musselshell River populations is approximately 60 km.

## MATERIALS AND METHODS

DNA samples were collected from 218 plants at 30 locations (13 cities containing ornamental plants and 17 wild populations) from eastern Montana, western North Dakota and Wyoming (Figure 1). Exact voucher locations are available from either author. A voucher specimen for each wild population or ornamental plant is stored at USDA/ARS/

NPARL, Sidney, MT. The ornamental plants were chosen mostly from those mentioned in Pearce and Smith (2003) and included 36 plants from Billings Ranch, Circle, Forsyth, Glendive, Hysham, Jordan, Kerr Ranch, Malta, Melstone, Roundup, Sidney, and St. Marie, MT. With the help of county weed-control personnel, we believe that we collected DNA from a majority of the extant ornamental plants in these regions, especially in the Musselshell River/Ft. Peck Lake drainage. The wild plant samples included in this study are from various sources and projects including earlier surveys of saltcedar genotypes in the USA (Gaskin and Schaal 2002). Intensively sampled populations (Howery's Island, Nelson Creek, and Devil's Creek, MT, and Lake Sakakawea, ND) are from a study of the influence of saltcedar genetic variation on performance of the classical biological control agent *Diorhabda elongata* Brulle. The remaining wild plants were sampled specifically for this project. The 182 wild plants were older (larger) plants from populations closest to ornamental plantings (2–50 km distant) or downstream populations. In all, from MT, ND, and WY, a total of 218 plants were analyzed for the chloroplast and nuclear DNA markers.

#### DNA Isolation, PCR Amplification, Sequencing and Analysis

Fresh, silica-dried tissue was used for DNA extraction. Genomic DNA was isolated using a modified CTAB method (Hillis et al. 1996). PCR amplification of the chloroplast intergenic region between the *trnS* (GCU) and *trnG* (UCC) genes utilized the primer pair trn S (GCU) (5'-GCCGCTTTAGTCCACTCAGC-3') and trn G (UCC) (5'-GAACGAATCACACTTTTACCAC-3') of Hamilton (1999). The cycling conditions were 95°C (2 min); 30 cycles of 95°C (1 min), 55°C (1 min), 72°C (2 min); and then 72°C (5 min). The fourth phosphoenolpyruvate carboxylase (*PepC*) intron nuclear region was amplified by PCR using the primer pair ppcx4f (5'-ACTCCACAGGATGAGATGAG-3') and ppcx5r (5'-GCAGCCATCATTCTAGCCAA-3') designed by J.G. from other taxa of the Caryophyllales listed in GenBank (cycling conditions were as above except that an annealing temperature of 52°C (1 min) was used). This primer pair amplifies DNA product for many angiosperm families, but in order to avoid double-banded PCR products, *Tamarix* specific *PepC* primers (with an annealing temperature of 50°C) were recently developed by J.G.: PPCL1 (forward) (5'-GTCCCTAAGTTTCTGCGTCG-3') and PPCL2 (reverse) (5'-CTTCAGGTGTTACTCTTGGG-3').

Table 1. GenBank accession numbers for DNA haplotype sequences found in ornamental and wild saltcedars (*Tamarix* spp.) from the eastern Montana region, USA.

| Chloroplast haplotype | GenBank accession # |
|-----------------------|---------------------|
| A                     | AF490798            |
| D                     | AF539998            |
| G                     | AF490782            |
| Nuclear haplotype     | GenBank accession # |
| 1                     | AY090385            |
| 2                     | AY090386            |
| 11                    | AY090395            |
| 12                    | AY090396            |
| 14                    | AY090398            |
| 15                    | AY090399            |
| 28                    | AY090412            |
| 50                    | AY090434            |
| 51                    | AY090435            |
| 53                    | AY090437            |
| 55                    | AY090439            |
| 56                    | AY090440            |

To survey cpDNA variation without sequencing each individual, we took advantage of a known single nucleotide polymorphism in the intergenic region between the *trnS* (GCU) and *trnG* (UCC) genes. *Tamarix ramosissima* and *T. chinensis* contain an allele at that position that does not exist in other naturalized *Tamarix* species (Gaskin and Schaal 2003). DNA samples from all plants were amplified using the mismatch amplification mutation assay (MAMA) of Cha et al. (1992), which can distinguish between sequences that vary at one specific nucleotide. In this method, the base at the 3' end of the primer is designed to anneal to the sequence at the polymorphic site, and the penultimate 3' base is a mismatch for all known sequences, causing the primer to anneal to specific sequences only (in this case, *T. ramosissima* and *T. chinensis* but not other naturalized *Tamarix* spp.). The DNA sequences of other naturalized species do not have a location for the primer to anneal to, and should not amplify a PCR product. The mismatch primer was trnG-S 510R (5'-ACTTCTTTCCGGTTCGCATTTTCA-3'), and it was paired with the trn G (UCC) primer mentioned above. All plants that yielded no PCR product (those that did not have the correct DNA sequence at that primer annealing site) were also sequenced to verify that they were not *T. ramosissima* or *T. chinensis*, using the protocol below, for the cpDNA region (76 plants). An additional, randomly-chosen 17 plants that yielded PCR product and four plants that yielded weak PCR product (all putatively *T. ramosissima* and *T. chinensis*) were

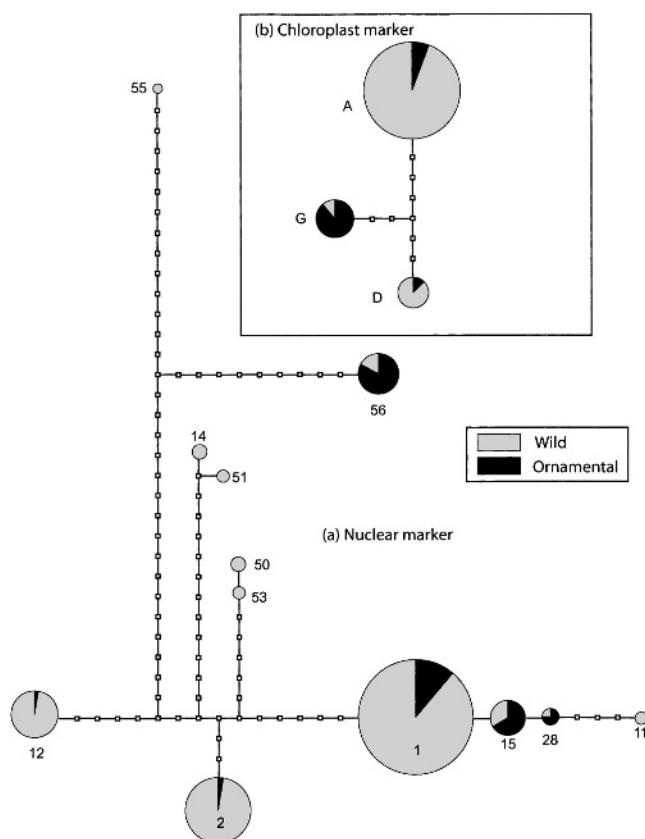


Figure 2. The nuclear *PepC* fourth intron (a) and chloroplast *trnS-trnG* intergenic spacer (b) haplotype networks based on 218 wild and ornamental *Tamarix* in the eastern Montana region, USA. Circles with numbers or letters represent haplotypes (alleles) recovered. The small empty boxes represent intermediate haplotypes not recovered in this analysis. Lines separating the haplotype boxes or circles represent a single point mutation or insertion/deletion event. The size of the haplotype circle is proportional to haplotype frequency. Gray and black portions of circles represent frequency of the haplotype found in wild and ornamental plants, respectively.

sequenced to verify the MAMA results. In total, 97 plant cpDNA haplotypes were determined solely from the MAMA analysis, and the remaining plant cpDNA and nuclear haplotypes were determined from sequence data.

For sequencing, a 50- $\mu$ l reaction was performed for each individual, and PCR products were purified using the QIAquick PCR Purification kit (Qiagen). Purified templates were sequenced using the PCR primers above on a CEQ 2000XL (Beckman) automated sequencer. Nuclear haplotypes were inferred from heterozygous genotypic sequences using "haplotype subtraction" (Clark 1990), and to check our haplotype inferences, we cloned and sequenced PCR product from plants representing each inferred haplotype (12 plants total). Clones

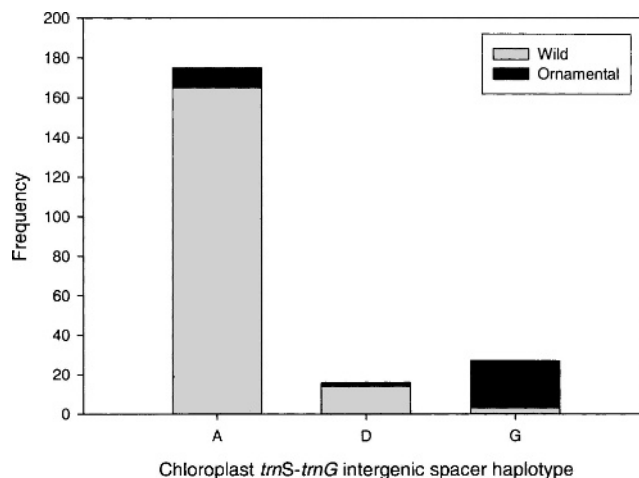


Figure 3. Frequency of chloroplast haplotypes for wild and ornamental *Tamarix* in the eastern Montana region, USA.

were created with the pGEM-T Vector System II (Promega) and then sequenced as above. Haplotype sequences found in this study are available on GenBank (accession numbers in Table 1) and were manually aligned using the software Se-Al (Rambaut 1996). The alignment is available upon request from the first author. Most parsimonious haplotype networks were created by hand. Insertion/deletion events, no matter what size, were treated as one mutational event (one evolutionary step).

Log-likelihood ratio tests were used to compare haplotype and genotype frequencies between the ornamental and wild saltcedar populations using the FREQ procedure of SAS (SAS Institute Inc. 2000). The EXACT option of the FREQ procedure was used to invoke computation of exact *p*-values because a large percentage of the cells in the  $2 \times N$  tables underlying these tests (where *N* is the number of haplotypes or genotypes) had expected counts of less than 5. Monte Carlo approximations to the exact *p*-values based on  $10^5$  resamples of the original data were made in cases where *N* exceeded 3. Similar tests were used to compare the genotype frequencies of the wild saltcedar populations to expected genotype frequencies following one generation of random mating, self-fertilization, or clonal reproduction among the ornamental plants. Expected genotype frequencies under the random mating and self-fertilization scenarios were calculated assuming standard Mendelian inheritance for the nuclear gene and maternal inheritance for the chloroplast gene. Expected genotype frequencies under the clonal reproduction scenario are the same as the observed genotype frequencies of the ornamental plants. GeneClass 2.0 (Piry et al. 2004) was

Table 2. Haplotype frequencies in ornamental and wild saltcedars (*Tamarix* spp.) from the eastern Montana region, USA.

|                       |                  | N <sup>b</sup> | Chloroplast hap-<br>lotype frequency |    |    | Nuclear haplotype frequency |    |    |    |    |    |    |    |    |    |    |    |
|-----------------------|------------------|----------------|--------------------------------------|----|----|-----------------------------|----|----|----|----|----|----|----|----|----|----|----|
| Location <sup>a</sup> |                  |                | A                                    | D  | G  | 1                           | 2  | 11 | 12 | 14 | 15 | 28 | 50 | 51 | 53 | 55 | 56 |
| Ornamental            |                  |                |                                      |    |    |                             |    |    |    |    |    |    |    |    |    |    |    |
| 1                     | Billings Ranch   | 2              |                                      |    | 2  | 2                           |    |    |    |    |    |    |    |    |    |    | 2  |
| 2                     | Circle           | 6              | 5                                    |    | 1  | 1                           |    |    |    |    | 9  | 1  |    |    |    |    | 1  |
| 3                     | Forsyth          | 5              |                                      |    | 5  | 5                           |    |    |    |    |    |    |    |    |    |    | 5  |
| 4                     | Glendive         | 1              |                                      | 1  |    | 2                           |    |    |    |    |    |    |    |    |    |    |    |
| 5                     | Hysham           | 3              | 2                                    |    | 1  | 1                           |    |    |    |    | 4  |    |    |    |    |    | 1  |
| 6                     | Jordan           | 1              |                                      |    | 1  |                             | 1  |    |    |    |    |    |    |    |    |    | 1  |
| 7                     | Kerr Ranch       | 1              |                                      |    | 1  | 1                           |    |    |    |    |    |    |    |    |    |    | 1  |
| 8                     | Malta            | 2              | 1                                    | 1  |    | 2                           | 1  |    | 1  |    |    |    |    |    |    |    |    |
| 9                     | Melstone         | 1              | 1                                    |    |    | 1                           |    |    |    |    |    | 1  |    |    |    |    |    |
| 10                    | Roundup          | 5              |                                      |    | 5  | 5                           |    |    |    |    |    |    |    |    |    |    | 5  |
| 11                    | Sidney           | 4              | 1                                    |    | 3  | 3                           |    |    |    |    | 1  | 1  |    |    |    |    | 3  |
| 12                    | St. Marie        | 4              |                                      |    | 4  | 4                           |    |    |    |    |    |    |    |    |    |    | 4  |
| 13                    | Winnett          | 1              |                                      |    | 1  | 1                           |    |    |    |    |    |    |    |    |    |    | 1  |
| Ornamental total      |                  | 36             | 10                                   | 2  | 24 | 28                          | 2  | 0  | 1  | 0  | 14 | 3  | 0  | 0  | 0  | 0  | 24 |
| Wild                  |                  |                |                                      |    |    |                             |    |    |    |    |    |    |    |    |    |    |    |
| 14                    | Boysen Reservoir | 7              | 6                                    | 1  |    | 7                           | 6  |    |    |    |    |    | 1  |    |    |    |    |
| 15                    | Broadus          | 6              | 6                                    |    |    | 7                           | 4  |    | 1  |    |    |    |    |    |    |    |    |
| 16                    | Devil's Creek    | 33             | 26                                   | 7  |    | 38                          | 16 |    | 11 |    |    |    | 1  |    |    |    |    |
| 17                    | Five Mile Creek  | 7              | 7                                    |    |    | 3                           | 5  |    | 1  | 3  |    |    |    | 1  |    |    | 1  |
| 18                    | Farwest Boatramp | 4              | 4                                    |    |    | 5                           | 1  |    |    |    | 2  |    |    |    |    |    |    |
| 19                    | Hardin           | 3              | 3                                    |    |    | 6                           |    |    |    |    |    |    |    |    |    |    |    |
| 20                    | Hell Creek       | 10             | 9                                    | 1  |    | 9                           | 3  |    | 1  |    | 2  |    | 1  | 1  |    | 1  | 2  |
| 21                    | Howery's Island  | 34             | 33                                   | 1  |    | 46                          | 13 |    | 7  |    |    |    |    |    | 1  |    | 1  |
| 22                    | Highway 500      | 3              | 3                                    |    |    | 4                           | 2  |    |    |    |    |    |    |    |    |    |    |
| 23                    | Kinsey           | 2              | 2                                    |    |    | 3                           | 1  |    |    |    |    |    |    |    |    |    |    |
| 24                    | Lovell           | 10             | 10                                   |    |    | 12                          | 5  |    | 1  |    | 1  |    |    |    | 1  |    |    |
| 25                    | Melstone         | 8              | 7                                    | 1  |    | 14                          | 1  |    | 1  |    |    |    |    |    |    |    |    |
| 26                    | N. Melstone      | 8              | 7                                    |    | 1  | 10                          | 4  |    | 1  |    |    | 1  |    |    |    |    |    |
| 27                    | Nelson Creek     | 30             | 28                                   | 2  |    | 34                          | 12 | 2  | 10 |    | 1  |    |    |    |    |    | 1  |
| 28                    | Powder River     | 5              | 5                                    |    |    | 8                           | 2  |    |    |    |    |    |    |    |    |    |    |
| 29                    | Lake Sakakawea   | 7              | 4                                    | 1  | 2  | 10                          | 1  |    | 3  |    |    |    |    |    |    |    |    |
| 30                    | Laurel           | 5              | 5                                    |    |    | 7                           | 1  |    | 1  |    | 1  |    |    |    |    |    |    |
| Wild total            |                  | 182            | 165                                  | 14 | 3  | 223                         | 77 | 2  | 38 | 3  | 7  | 1  | 3  | 2  | 2  | 1  | 5  |
| Combined total        |                  | 218            | 175                                  | 16 | 27 | 251                         | 79 | 2  | 39 | 3  | 21 | 4  | 3  | 2  | 2  | 1  | 29 |

<sup>a</sup> Location numbers correspond to numbers on map (Figure 1).<sup>b</sup> N = number of plants sampled at each location.

used to calculate assignment scores using the allele frequency criterion of Paetkau et al. (1995). The assignment score for each genotype is the likelihood value for an individual with that genotype originating in the population it was found in divided by the sum of the likelihood values for that genotype originating in the ornamental and wild populations (multiplied by 100, see Piry et al. 2004 for details). An assignment score greater than 50 indicates that an individual is more likely to have originated in the population where it was found than the other population. Conversely, an assignment score less than 50 indicates that an individual is more likely to

have originated from a population other than the one in which it was found.

## RESULTS AND DISCUSSION

### Chloroplast Marker

The chloroplast *trn* S (GCU) - *trn* G (UCC) region has an aligned length of 1001 base pairs (bp), with eight base substitutions, one mono-nucleotide repeat region (with two states), and one di-nucleotide repeat region (with two states), for a total of 10 (1.1%) variable sites. Three distinct chloroplast

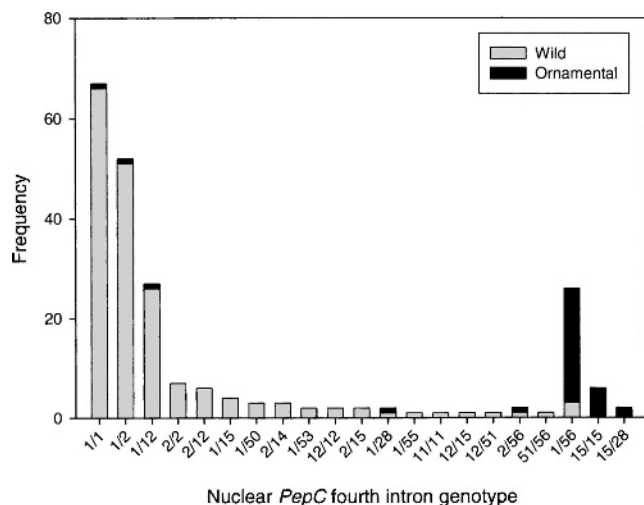


Figure 4. Frequency of nuclear haplotypes for wild and ornamental *Tamarix* in the eastern Montana region, USA.

haplotypes (alleles) were recovered from 218 plants: A, D, and G. A haplotype network with the relative frequency of occurrence, number of mutational steps between haplotypes, and status of plants (ornamental or wild) is shown in Figure 2.

Chloroplast haplotype frequencies differed significantly between ornamental and wild plants (likelihood ratio test statistic ( $LR$ ) = 87.8,  $df$  = 2,  $P$  < 0.0001). Although each of the three haplotypes was found in both ornamental and wild plants, 91% of the wild plants were haplotype A and 67% of the ornamental plants were haplotype G (Figure 3, Table 2). In previous studies, haplotype A was found commonly ( $n$  = 46, 78%) in native Eurasian and naturalized USA *T. ramosissima* and *T. chinensis* plants (Gaskin 2003) but also in native specimens of *T. korolkowii* Regel et Smalh., *T. smyrnensis* Bge., and *T. aralensis* Bge. (Gaskin and Schaal 2003). The D haplotype was previously found in various species, including native *T. elongata* Ledeb., *T. boveana* Bge., *T. meyeri* Boiss., and consistently in native and naturalized *T. parviflora* plants (Gaskin and Schaal 2003; Gaskin unpublished data). The D haplotype was also previously rarely found ( $n$  = 1, 2%) in naturalized *T. ramosissima* samples, and the G haplotype was found in all previous samples of ornamental saltcedars ( $n$  = 5, 100%), rarely ( $n$  = 2, 7%) in USA naturalized saltcedar plants, and never in Eurasia (Gaskin 2003).

#### Nuclear Marker

The *PepC* intron region has an aligned length of 901 bp, with 71 base substitutions, two mononucleotide repeat regions (with two variable states each), and three insertion/deletion events (indels) of

1, 6, and 15 bp (the indels are at different loci and do not overlap) for a total of 76 (8.4%) variable sites. Twelve distinct nuclear haplotypes, in 21 different genotypic combinations, were found in the 218 plants sampled. A haplotype network with the relative frequency of occurrence, number of mutational steps between haplotypes, and status of plants (ornamental or wild) is shown in Figure 2. The number of variable sites (76) and the number of steps on the network (86) are not equal due to homoplasy (mutations found in more than one place on the haplotype network).

The *PepC* haplotype and genotype frequencies differed significantly between the ornamental and wild plants (haplotype frequencies:  $LR$  = 129.3,  $df$  = 11,  $P$  < 0.0001; genotype frequencies:  $LR$  = 142.4,  $df$  = 20,  $P$  < 0.0001, Figure 4). The most common nuclear genotypes of the 182 wild plants in this study were 1/1 ( $n$  = 66, 36%), 1/2 ( $n$  = 51, 28%), and 1/12 ( $n$  = 26, 14%). These are also three of the four most common nuclear genotypes found throughout the western USA wild populations in an earlier study, but the *T. chinensis* genotype 2/2 was not found as frequently in this study ( $n$  = 7, 4%) as it was in the rest of the western USA ( $n$  = 30, 19%) (Gaskin and Schaal 2002). Genotypes 1/1 and 2/2 are commonly found in central Asia in *T. ramosissima* and eastern Asia in *T. chinensis* plants, respectively. Genotype 1/12 was also relatively common in the rest of the USA in the earlier study ( $n$  = 14, 9%) but was not found in Asia. The haplotype 12 was only found twice (1%) in Asia in a 12/12 homozygote in Azerbaijan (Gaskin and Schaal 2002). The rest of the wild nuclear genotypes from this study are at frequencies of less than 3%.

Genotype 1/56 ( $n$  = 23, 64%) was the most common genotype in the ornamental plants. It was also found in three (1.6%) of the wild plants. Two genotypes, 15/15 ( $n$  = 6, 17%) and 15/28 ( $n$  = 2, 6%), were found only in ornamental plants. Five other genotypes were observed once in the ornamental plants but were also observed in the wild plants (Figure 4).

Ornamental saltcedars that are morphologically indistinguishable represent various nuclear genotypes. In earlier work (Gaskin and Schaal 2002), a commercially available *T. ramosissima* 'Pink Cascade' plant contained the nuclear genotype 2/56. Haplotype 2 is found in all *T. chinensis* plants from Asia (western China), but the origin of haplotype 56 is unknown. Haplotype 56 was found in less than 1% ( $n$  = 3) plants in an earlier study of the wild USA plants (Gaskin and Schaal 2002) and was never found in a survey of 114 Asian *Tamarix* plants containing various species morphologically



Table 3. Combined chloroplast-nuclear genotype frequencies (as percentages) observed in wild and ornamental saltcedars (*Tamarix* spp.) in the eastern Montana region, USA. Expected genotype frequencies following one generation of random mating or self-fertilization of the ornamental plants are also shown. Expected genotype frequencies following clonal reproduction of the ornamental plants are the same as those observed in the ornamental plants. Genotype frequencies greater than 5% are shown in bold<sup>a</sup>.

| Chloroplast-nuclear genotype                   | Observed or expected genotype frequencies |   |  |  |
|--|---|---|--|--|
|  | Wild plants (observed, N=182)             | Random mating of ornamental plants (expected) | Self-fertilization of ornamental plants (expected) | Ornamental plants (observed, N=36) Clonal reproduction of ornamental plants (expected) |
| A-1/1  | <b>34.07</b>                              | 1.08  | 1.39   | 0.00   |
| A-1/2  | <b>25.27</b>                              | 0.62  | 1.39   | 2.78   |
| A-1/12   | <b>10.99</b>                              | 0.04  | 0.00   | 0.00   |
| A-2/2  | 3.85                                      | 0.04  | 0.69   | 0.00   |
| A-2/12   | 3.30                                      | 0.02  | 0.00   | 0.00   |
| D-1/12   | 2.75                                      | 0.60  | 1.39   | 2.78   |
| D-1/1  | 2.20                                      | 1.62  | 3.47   | 2.78   |
| A-1/15   | 2.20                                      | <b>8.10</b>                                   | 0.00   | 0.00   |
| D-1/2  | 2.20                                      | 0.12  | 0.00   | 0.00   |
| A-1/56   | 1.65                                      | 0.93  | 0.00   | 0.00   |
| A-1/50   | 1.65                                      | 0.00  | 0.00   | 0.00   |
| A-2/14   | 1.65                                      | 0.00  | 0.00   | 0.00   |
| A-2/15   | 1.10                                      | 0.81  | 0.00   | 0.00   |
| A-1/53   | 1.10                                      | 0.00  | 0.00   | 0.00   |
| A-12/12  | 1.10                                      | 0.00  | 0.00   | 0.00   |
| G-1/2  | 0.55                                      | 1.43  | 0.00   | 0.00   |
| G-1/28   | 0.55                                      | 1.33  | 0.00   | 0.00   |
| A-2/56   | 0.55                                      | 0.46  | 0.00   | 0.00   |
| G-1/12   | 0.55                                      | 0.44  | 0.00   | 0.00   |
| A-12/15  | 0.55                                      | 0.27  | 0.00   | 0.00   |
| A-1/55   | 0.55                                      | 0.00  | 0.00   | 0.00   |
| A-11/11  | 0.55                                      | 0.00  | 0.00   | 0.00   |
| A-51/56  | 0.55                                      | 0.00  | 0.00   | 0.00   |
| D-12/51  | 0.55                                      | 0.00  | 0.00   | 0.00   |
| G-1/56   | 0.00                                      | <b>23.61</b>                                  | <b>31.94</b>                                       | <b>63.89</b>   |
| A-15/15  | 0.00                                      | 3.78  | <b>18.06</b>                                       | <b>16.67</b>   |
| A-15/28  | 0.00                                      | 1.62  | 2.78   | <b>5.56</b>  |
| A-1/28   | 0.00                                      | 1.74  | 1.39   | 2.78   |
| G-2/56   | 0.00                                      | 1.39  | 1.39   | 2.78   |
| G-1/1  | 0.00                                      | <b>12.42</b>                                  | <b>15.97</b>                                       | 0.00   |
| G-56/56  | 0.00                                      | <b>11.11</b>                                  | <b>16.67</b>                                       | 0.00   |
| A-15/56  | 0.00                                      | <b>6.48</b>                                   | 0.00   | 0.00   |
| G-15/56  | 0.00                                      | <b>6.48</b>                                   | 0.00   | 0.00   |
| G-1/15   | 0.00                                      | <b>6.21</b>                                   | 0.00   | 0.00   |
| A-28/56  | 0.00                                      | 1.39  | 0.00   | 0.00   |
| D-1/56   | 0.00                                      | 1.39  | 0.00   | 0.00   |
| G-28/56  | 0.00                                      | 1.39  | 0.00   | 0.00   |
| D-1/15   | 0.00                                      | 0.81  | 0.00   | 0.00   |
| D-12/56  | 0.00                                      | 0.46  | 0.00   | 0.00   |
| G-12/56  | 0.00                                      | 0.46  | 0.00   | 0.00   |
| A-28/28  | 0.00                                      | 0.17  | 2.08   | 0.00   |
| G-2/2  | 0.00                                      | 0.04  | 0.69   | 0.00   |
| D-12/12  | 0.00                                      | 0.02  | 0.69   | 0.00   |
| Likelihood ratio test statistic <sup>b</sup> : |   | 416.8   | 429.0  | 444.3  |
| df:  |   | 39  | 33   | 28   |
| P:   |   | < 0.0001                                      | < 0.0001   | < 0.0001   |

<sup>a</sup> Genotypes not observed and with expected frequencies less than 0.28% following one generation of random mating or self-fertilization of the ornamental plants are not shown.

<sup>b</sup> The likelihood ratio test statistics test the hypotheses that the observed genotype frequencies in wild plants are equal to the expected genotype frequencies following one generation of random mating, self-fertilization, or clonal reproduction of the ornamental plants.

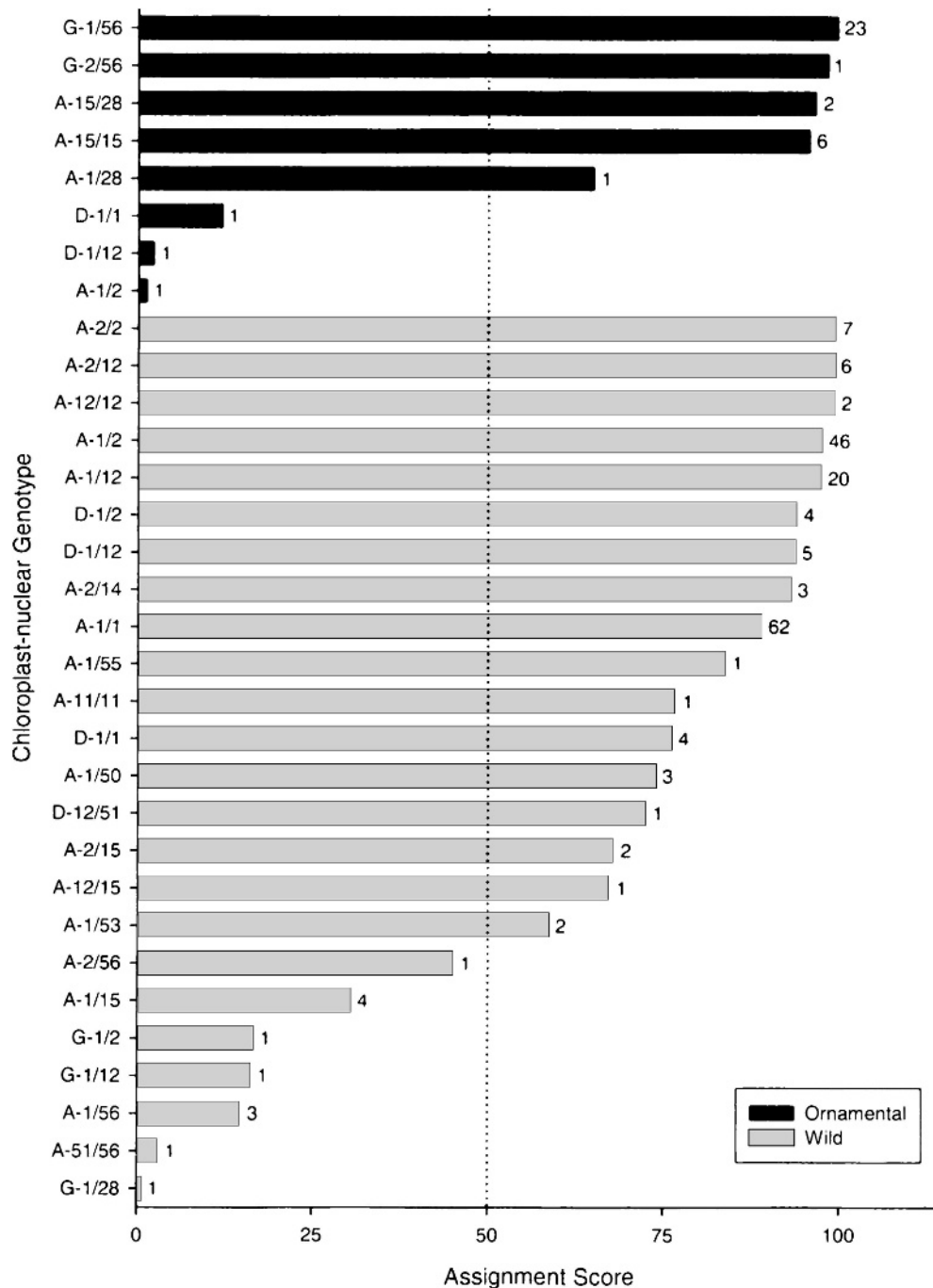


Figure 5. Assignment scores of combined chloroplast-nuclear genotypes for ornamental and wild *Tamarix* in the eastern Montana region, USA. An assignment score greater than 50 indicates that individuals with that genotype are more likely to have originated in the population where found than the other population. Conversely, an assignment score less than 50 indicates those individuals are more likely to have originated from the population other than where found. Numbers at right end of bars indicate frequency of genotype.

Table 4. Combined chloroplast-nuclear genotype frequencies (as percentages) observed in wild and ornamental saltcedars (*Tamarix* spp.) on the Musselshell River, Montana, USA. Expected genotype frequencies following one generation of random mating or self-fertilization of the ornamental plants are also shown. Expected genotype frequencies following clonal reproduction of the ornamental plants are the same as those observed in the ornamental plants. Genotype frequencies greater than 10% are shown in bold.

| Chloroplast-nuclear genotype                   | Observed or expected genotype frequencies |   |  |   |
|--|---|---|--|---|
|  | Wild plants (observed, N=19)              | Random mating of ornamental plants (expected) | Self-fertilization of ornamental plants (expected) | Ornamental plants (observed, N=6) Clonal reproduction of ornamental plants (expected) |
| A-1/1  | <b>47.37</b>                              | 4.17  | 4.17   | 0.00  |
| A-1/2  | <b>26.32</b>                              | 0.00  | 0.00   | 0.00  |
| A-1/12   | <b>10.53</b>                              | 0.00  | 0.00   | 0.00  |
| G-1/28   | 5.26                                      | 3.47  | 0.00   | 0.00  |
| A-2/2  | 5.26                                      | 0.00  | 0.00   | 0.00  |
| D-1/1  | 5.26                                      | 0.00  | 0.00   | 0.00  |
| G-1/56   | 0.00                                      | <b>38.19</b>                                  | <b>41.67</b>                                       | <b>83.33</b>  |
| A-1/28   | 0.00                                      | 4.86  | 8.33   | <b>16.67</b>  |
| G-1/1  | 0.00                                      | <b>20.83</b>                                  | <b>20.83</b>                                       | 0.00  |
| G-56/56  | 0.00                                      | <b>17.36</b>                                  | <b>20.83</b>                                       | 0.00  |
| A-1/56   | 0.00                                      | 3.47  | 0.00   | 0.00  |
| A-28/56  | 0.00                                      | 3.47  | 0.00   | 0.00  |
| G-28/56  | 0.00                                      | 3.47  | 0.00   | 0.00  |
| A-28/28  | 0.00                                      | 0.69  | 4.17   | 0.00  |
| Likelihood ratio test statistic <sup>a</sup> : |   | 44.8  | 47.5   | 52.7  |
| df:  |   | 12  | 10   | 7   |
| P:   |   | < 0.0001                                      | < 0.0001   | < 0.0001  |

<sup>a</sup> The likelihood ratio test statistics test the hypotheses that the observed genotype frequencies in wild plants are equal to the expected genotype frequencies following one generation of random mating, self-fertilization, or clonal reproduction of the ornamental plants.

similar to *T. ramosissima* (Gaskin and Schaal 2002). Haplotype 56 is genetically very distant from the common *T. ramosissima* (1) and *T. chinensis* (2) haplotypes (36 mutations and 32 mutations respectively, see haplotype network: Figure 2). It is likely that the 1/56 and 2/56 genotypes are hybrids of *T. ramosissima* or *T. chinensis*, respectively, with an as yet unidentified *Tamarix* species.

### Combined Genotypes

The most common combined chloroplast and nuclear genotype found in ornamental plants is G-1/56 (64%), and for wild plants it is A-1/1 (34%) (Table 3). Combined nuclear/chloroplast genotypes have not been reported for any other native or wild populations in earlier studies. The expected frequencies of genotypes in propagule populations derived from ornamental plants under clonal reproduction, random mating, and self-fertilization scenarios are significantly different from observed genotype frequencies of wild plants ( $P < 0.0001$  in all cases, Table 3).

Assignment scores greater than 50% were found for 17 (71%) of the wild combined genotypes

(representing 170 (93%) of the wild plants) and five (63%) of the ornamental combined genotypes (representing 32 (92%) of the ornamental plants, Figure 5). Three ornamental plants with low assignment scores ( $< 20\%$ ) to the ornamental population had genotypes D-1/1, D-1/12 and A-1/2. These were the only combined genotypes found in both the ornamental and wild populations, with the A-1/2 genotype being the second most common genotype in the wild. Seven wild plants with low assignment scores to the wild population had genotypes G-1/2, G-1/12, A-1/56, A-51/56 and G-1/28. None of the latter genotypes were found in the ornamental population.

### Musselshell River

We found six ornamental plants in Melstone and Roundup, MT. Nearby and downstream plants ( $n = 19$ ) on the Musselshell River contained combined genotypes that were all different from those found in the ornamental plants (Table 4). The expected frequencies of genotypes in propagule populations derived from these ornamental plants under the clonal reproduction, random mating, and self-

fertilization scenarios are significantly different from the observed genotype frequencies of wild plants ( $P < 0.0001$  in all cases, Table 4).

### CONCLUSIONS

Ornamental saltcedars in the eastern Montana region are a diverse group of eight different genotypes, involving combinations of haplotypes commonly found in multiple *Tamarix* species. In general, chloroplast-nuclear genotypes found in ornamental *Tamarix* are dissimilar from genotypes commonly found in nearby wild populations. Hypothetical propagule populations derived from these ornamental plants under clonal reproduction, random mating, or self-fertilization scenarios have expected genotype frequencies that differ significantly from what is observed in the wild. Assignment tests also indicate that the majority of wild genotypes originated from other wild plants, not from ornamental plants. The Musselshell River populations could not have been started entirely from the nearest ornamental plantings that we genotyped, and hypothetical propagule populations derived from these ornamental plants also have expected genotype frequencies that differ significantly from what is observed in nearby wild populations. Haplotypes unique to the wild populations must have come from other dispersal sources, existing ornamental plants that we did not locate, or ornamental plants that no longer exist.

The ornamental and wild populations have marked genetic differences, and ornamental plants may not be prolific seed producers, but in an ongoing study of *Tamarix* seed viability (Kazmer and Gaskin, unpublished data), an ornamental plant produced a small percentage of viable seed through self-fertilization, and another ornamental plant contained viable seeds, potentially through outcrossing. These preliminary results suggest that ornamental plants, even solitary ones, cannot be excluded as contributors to wild populations. All chloroplast and nuclear haplotypes, and all but two nuclear genotype combinations (15/15 and 15/28) found in the ornamental plants, were found at some frequency in wild populations. The possibility of pollen or propagule movement from an ornamental population to a wild population, even on a limited basis, argues against the ecological safety of ornamental saltcedar plants.

A further argument against the use of ornamental saltcedar is the possible movement of pollen or propagules from the wild population to the ornamental population. Anecdotal evidence exists of

plants that have been removed from wild populations and planted as ornamentals. Three ornamental plants in our study had chloroplast-nuclear genotypes also found in the wild populations, and one of these genotypes (A-1/2) is common in the wild. Nuclear haplotypes 1 and 2, and chloroplast haplotype A, are found in some ornamental plants, and if it is possible for the plants to either cross-pollinate with other ornamental plants containing these haplotypes or to self-fertilize, genotypes A-1/1, A-1/2, and A-2/2 could be produced, which are the most common genotypes found in the wild USA populations (Gaskin and Schaal 2002). Without pedigree information or genotyping, commercial cultivars cannot be confidently distinguished from plants propagated from wild cuttings. Thus, while plants with the common ornamental genotypes may contribute little to wild populations, ornamental plants derived from wild populations could make considerable contributions to new and extant wild populations.

### ACKNOWLEDGMENTS

G. Keasley, A. Adler, S. Bockness, J. Sparks, C. Clark, J. Cramer, R. Stellflug, and other county weed-coordinators kindly assisted in locating or collecting plant samples. T. Unruh, F. Ryan, and two anonymous reviewers provided helpful comments on earlier versions of the manuscript. This research was made possible in part by financial support from the U.S. Department of the Interior, Bureau of Land Management and Bureau of Indian Affairs.

### LITERATURE CITED

- Anderson, N. O. and P. D. Ascher. 1993. Male and female fertility of loosestrife (*Lythrum*) cultivars. *Journal of the American Society for Horticultural Science* 118:851–858.
- Baum, B. R. 1978. The Genus *Tamarix*. Israel Academy of Sciences and Humanities, Jerusalem, Israel.
- Brotherson, J. D. and D. Field. 1987. *Tamarix*: impacts of a successful weed. *Rangelands* 3:110–112.
- Cha, R. S., H. Zarbl, P. Keohavong, and W. G. Thilly. 1992. Mismatch amplification mutation assay (MAMA): application to the c-H-ras gene. *PCR Methods and Applications* 2:14–20.
- Chapman, M. and J. Burke. 2006. Letting the gene out of the bottle: the population genetics of genetically modified crops. *New Phytologist* 170:429–443.
- Clark, A. G. 1990. Inference of haplotypes from PCR-amplified samples of diploid populations. *Molecular Biology and Evolution* 7:111–112.
- Cornuet, J. M., S. Piry, G. Luikart, A. Estoup, and M. Solignac. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics* 153:1989–2000.
- Crins, W. J. 1989. The Tamaricaceae of the southeastern United States. *Journal of the Arnold Arboretum* 70:403–425.

- Ellstrand, N. C. 2003. *Dangerous Liaisons? When Cultivated Plants Mate with Their Wild Relatives*. John Hopkins University Press, Baltimore, MD, USA.
- Gaskin, J. F. 2003. Molecular systematics and the control of invasive plants: A case study of *Tamarix* (Tamaricaceae). *Annals of the Missouri Botanical Garden* 90:109–118.
- Gaskin, J. F. and B. A. Schaal. 2002. Hybrid *Tamarix* widespread in US invasion and undetected in native Asian range. *Proceedings of the National Academy of Sciences of the United States of America* 99:11256–11259.
- Gaskin, J. F. and B. A. Schaal. 2003. Molecular phylogenetic investigation of US invasive *Tamarix*. *Systematic Botany* 28:86–95.
- Hamilton, M. 1999. Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Molecular Ecology* 8:521–523.
- Hillis, D. M., B. K. Mable, A. Larson, S. K. Davis, and E. A. Zimmer. 1996. *Molecular Systematics*. Sinauer, Sunderland, MA, USA.
- Horton, J. S. 1964. Notes on the introduction of deciduous Tamarisk [into the U.S.A.]. United States Department of Agriculture, Rocky Mountain Forest and Range Experiment Station, Ft. Collins, CO, USA. Research Note RM-16.
- Jenczewski, E., J. Ronfort, and A. Chevre. 2003. Crop-to-wild gene flow, introgression and possible fitness effects of transgenes. *Environmental Biosafety Research* 2:9–24.
- Lindgren, C. J. and R. T. Clay. 1993. Fertility of “Morden Pink” *Lythrum virgatum* L. transplanted into wild stands of *L. salicaria* in Manitoba. *HortScience* 28:954.
- Mack, R. N. and M. Erneberg. 2002. The United States naturalized flora: Largely the product of deliberate introductions. *Annals of the Missouri Botanical Garden* 89:176–189.
- Ottensbreit, K. A. and R. J. Staniforth. 1994. Crossability of naturalized and cultivated *Lythrum* taxa. *Canadian Journal of Botany* 72:337–341.
- Paetkau, D., W. Calvert, I. Stirling, and C. Strobeck. 1995. Microsatellite analysis of population-structure in Canadian polar bears. *Molecular Ecology* 4:347–354.
- Panstruga, R., A. Seiler, H.-J. Hirsch, and F. Kreuzaler. 1995. Genomic structure of a phosphoenolpyruvate carboxylase gene from potato (*Solanum tuberosum*). *Plant Physiology* 109:1126.
- Pearce, C. M. and D. G. Smith. 2003. Saltcedar: distribution, abundance, and dispersal mechanisms, northern Montana, USA. *Wetlands* 23:215–228.
- Piry, S., A. Alapetite, J.-M. Cornuet, D. Paetkau, L. Baudouin, and A. Estoup. 2004. GeneClass2: A software for genetic assignment and first generation migrant detection. *Journal of Heredity* 95:536–539.
- Rambaut, A. 1996. Se-Al Sequence Alignment Editor (<http://evolve.zoo.ox.ac.uk/software.html?id=seal>), Oxford, UK.
- Reichard, S. H. 1997. Prevention of invasive plant introductions on national and local levels. p. 215–227. *In* J. A. Luken and J. A. Thieret (eds.) *Assessment and Management of Plant Invasions*. Springer, New York, NY, USA.
- Rice, P. M. 2005. INVADERS Database System (<http://invader.dbs.umt.edu>). Division of Biological Sciences. University of Montana, Missoula, MT, USA.
- Ríos, J. L. V. and J. F. E. García. 1998. Catálogo de Malezas de México. Universidad Nacional Autónoma de México y el Fondo de Cultura Económica, México, DF.
- Robinson, T. W. 1965. Introduction, spread, and aerial extent of saltcedar (*Tamarix*) in the western states. U. S. Geological Survey Professional Paper 491-A.
- SAS Institute Inc. 2000. SAS OnlineDoc, Version 8. SAS Institute, Inc., Cary, NC, USA.
- Stenquist, S. M. 2000. Saltcedar integrated weed management and the Endangered Species Act. p. 487–504. *In* N. R. Spencer (ed.) *Proceedings of the 10th International Symposium on Biological Control of Weeds*. Montana State University, Bozeman, MT, USA.
- Wiedenmann, J., A. Baumstark, T. L. Pillen, A. Meinesz, and W. Vogel. 2001. DNA fingerprints of *Caulerpa taxifolia* provide evidence for the introduction of an aquarium strain into the Mediterranean Sea and its close relationship to an Australian population. *Marine Biology* 138:229–234.
- Wilgus, F. and K. Hamilton. 1962. Germination of salt cedar. *Weeds* 10:332–333.

Manuscript received 9 February 2006; revision received 27 May 2006; accepted 23 August 2006.